CLAIMS

1. A method in a process for the determination of an analyte in a sample involving utilizing biospecific affinity reactions, and comprising the following steps:

i. forming a complex comprising:
Reactant I---Analyte'---Reactant*, where
a. Reactant* and Reactant I exhibit biospecific affinity to the analyte,
b. Reactant* is analytically detectable,
c. Analyte' is the analyte or an analyte related reactant, and subsequently

ii. determining the detectable signal from Reactant* in the complex (sample value), and

iii. obtaining the amount of analyte in the sample by comparing the sample value with one or more calibrator values, each of which corresponds to a standard amount of analyte,

characterized in that before the determination of the calibrator value, either (i) the calibrator or (ii) a binder for the calibrator has been bound to a matrix, and when a binder for the calibrator has been bound to the matrix, calibrator is added or calibrator predeposited in the matrix is released at the determination of calibrator value, and that the matrix is insoluble in the liquid medium in which binding of Reactant* to the calibrator occurs.

2. The method according to claim 1, ~~characterized in that~~ wherein calibrator has been bound to the matrix before the determination of calibrator value.

3. The method according to claim 1, ~~characterized in that~~ wherein a binder for the calibrator has been bound to the matrix before the determination of calibrator value.

4. The method according to claim 1 or 3, **characterized** in that said binder for the calibrator is one member of a specific binding pair, and that the other member of the specific binding pair is coupled or conjugated to the calibrator.

5. The method according any of claims 1 to 4, **characterized** in that the calibrator and the analyte have the ability to biospecifically bind to Reactant* via equivalent binding sites.

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6. The method according to any of claims 1-5, **characterized** in that

a. the matrix is a flow matrix exhibiting one or more calibrator zones (CZ1, CZ2, CZ3 etc.),

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b. (i) each calibrator zone comprises calibrator in an amount corresponding to a standard amount of analyte, or (ii) each calibrator zone contains calibrator binder, the amount of calibrator binder and the amount of calibrator corresponding to a standard amount of analyte, and

c. Reactant* is bound to the calibrator by transporting Reactant* through the calibrator zones.

7. The method according to claim 6, wherein ~~characterized in the~~ flow matrix is a lateral flow matrix.

8. The method according to claim 6 or 7, **characterized** in that

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a. two or more of the zones CZ1, CZ2, CZ3 etc. comprising calibrator or binder for the calibrator are located in the same process flow, at least two of the zones

corresponding to different standard amounts of analyte, and

b. transport of Reactant* for binding to matrix calibrator in the various CZ takes place via this process flow.

9. The method according to claim 6 or 7, **characterized** in that

a. separate calibrator zones (CZ) are located in separate process flows, and

b. transport of Reactant* for binding to calibrator in a calibrator zone CZ occurs via the respective process flow.

10. The method according to any of claims 8 or 9, **characterized** in that

a. the process flow and the process flows, respectively, lack a detection zone, and

b. the complex is formed in a detection zone in a process flow lacking a calibrator zone and being present in a matrix of the same type as the calibrator zones.

11. The method according to any of claims 1-5, **characterized** in that the matrix is a flow matrix, and in that, along one and the same process flow, there are

a. one or more calibrator zones (CZ), each of which exhibits a matrix calibrator or a matrix calibrator binder,

b. one or more detection zones, none of which coincides with any calibrator zone, and in which a Capturer is firmly anchored and is either Reactant I or a biospecific affinity reactant, which directly or indirectly is able to bind Reactant I biospecifically,

c. an application zone for Reactant*, $A_{R*}Z$, which is located upstream of said CZ and DZ and to which Reactant* may have been predeposited, and

d. an application zone for sample (A_SZ) which is located

i. upstream of or coinciding with a detection zone,

ii. downstream or upstream of or coinciding with $A_{R*}Z$ ($A_SZ/A_{R*}Z$), or

iii. upstream of, downstream of or coinciding with a calibrator zone,

wherein preferably the zone of application of sample (A_SZ) is located upstream of both detection and calibrator zones, and in that Reactant* is added to $A_{R*}Z$ if Reactant* is not pre-deposited, or buffer is added to $A_{R*}Z$ if Reactant* is pre-deposited, and sample is added to A_SZ, optionally premixed with Reactant* if A_SZ and $A_{R*}Z$ concide, such that analyte and Reactant* reach DZ at the same time, or such that analyte reaches DZ before Reactant*.

12. The method according to claim 11, wherein ~~characterized in that~~ the calibrator zone or zones (KZ) exhibit a calibrator binder, and that calibrator is pre-deposited upstream of the calibrator zone or zones.

13. The method according to claim 11 ~~or 12, characterized~~ wherein ~~that~~ the process flow comprises two or more of said calibrator zones.

14. The method according to claim 11 ~~or 12, characterized in that~~ wherein the process flow comprises one or two of said calibrator zones, and in that the level of analyte in the sample is obtained by:

a. having access to one or more separately obtained calibrator values, and

b. comparing a calibrator value for a calibrator zone (Positive Internal Calibrator = PIC), being located in the same process flow as said detection zone, with one or more of the separately obtained calibrator values,

c. adapting the measurement signal from the detection zone to the deviation of the measurement signal for PIC from the separate calibrator values, and subsequently

d. obtaining the level of analyte in the sample by comparing the adapted measurement signal from the detection zone with one or more of the separately obtained calibrator values,

or vice versa with respect to what has been adapted and compared in steps c) and d.

15. The method according to any of claims 11-14, **characterized** in that

a. A_SZ is (i) common to $A_{R*}Z$ (= $A_SZ/A_{R*}Z$) or (ii) is located upstream of $A_{R*}Z$, and

b. for alternative (i) sample is premixed with Reactant* before it is added to the common zone $A_SZ/A_{R*}Z$, or sample is being added to the common zone $A_SZ/A_{R*}Z$ containing predeposited Reactant*, and for alternative (ii) sample is added to A_SZ, which is located upstream of $A_{R*}Z$ which in turn comprises predeposited Reactant*.

16. The method according to any of claims 6-15, **characterized** in that Reactant* has particles as analytically detectable group, and/or calibrator or calibrator binder and/or Capturer, if there is a detection zone, is/are anchored to the matrix via particles.

17. The method according to any of claims 1-16, **characterized** in that the analyte is an antibody directed to Reactant I or to Reactant*, and

a. Reactant* is an antibody directed to the analyte and Reactant I is an antigen/hapten, when the analyte is an antibody directed to Reactant I, and

b. Reactant* is an antigen or a hapten and Reactant I an antibody directed to the analyte, when the analyte is an antibody directed to Reactant*.

18. The method according to any of claims 1-16, **characterized** in that the analyte is an antigen, and Reactant* and Reactant I are antibodies directed to the analyte.

19. The method according to any of claims 1-18, **characterized** in that the method is performed as a part of diagnosing allergy or autoimmune disease.

20. A device for transforming measured signal values of a complexed, analytically detectable reactant (= Reactant*) to real amounts of analyte in a sample, in connection with performing an analysis method which utilizes biospecific affinity reactions for the determination of the amount of analyte in a sample, to form complexes comprising Reactant* in an amount which is related to the amount of analyte in the sample, **characterized** in that the kit exhibits:

a flow matrix in which there is an area of process flow for the transport of Reactant*, and that there is in this area

i. one or more calibrator zones (CZ1, CZ2 etc.) comprising a calibrator, or binder for the calibrator, which is firmly anchored to the matrix, the amounts of calibrator or calibrator binder,

respectively, being different for at least two calibrator zones, and the calibrator exhibiting binding sites to which Reactant* is able to bind, when Reactant* is transported through a calibrator zone, and

ii. an application zone for Reactant* ($A_{R*}Z$) upstream of said one or more calibrator zones.

21. The device according to claim 20, ~~characterized in that~~ wherein a calibrator binder is firmly anchored in the matrix and that the device comprises calibrator predeposited upstream of the calibrator zone, for example in A_SZ.

22. The device according to claim 20 ~~or 21, characterized in~~ wherein the device comprises Reactant* predeposited in $A_{R*}Z$.

23. The device according to claim 20, ~~21 or 22, characterized in that~~ wherein the process flow comprises a detection zone (DZ) which is located downstream of or coinciding with $A_{R*}Z$ and comprises a firmly anchored Capturer via which Reactant* can bind to DZ, and a zone of application of sample (A_SZ) which is located upstream of or coincides with said DZ.

24. The device according to claim 23, ~~characterized in that~~ wherein $A_{R*}Z$ is located upstream of or downstream of or coincides with A_SZ, where upstream or downstream locations are preferred.

~~25.~~ The device according to any of claims 23-24, **characterized** ~~in that~~ the firmly anchored reactant (Capturer) has biospecific affinity to the ~~analyte~~ or to an analyte-related reactant.

26. The device according to any of claims 23-24, **characterized** in that the firmly anchored reactant (Capturer) has biospecific affinity to a second reactant which in turn has biospecific affinity to the analyte or to an analyte-related reactant.

27. The device according to any of claims 23-26, **characterized** in that said one or more calibrator zones are located upstream of DZ.

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28. The device according to any of claims 23-27, **characterized** in that A_SZ is located upstream of all calibrator zones.

29. A test kit, **characterized** in that the kit comprises a device according to any one claims 20-28.

a 30. The kit according to claim 29, ~~**characterized in that**~~ wherein the kit comprises Reactant*.

a 31. The kit according to claim 29 ~~or 30, **characterized** in that~~ wherein the kit comprises calibrator when said device has calibrator binder bound to the matrix.

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